Journal of Medical Virology 61:143–149 (2000)

Genetic Characterization of Adenovirus Strains Isolated From Patients With Acute Conjunctivitis in the City of São Paulo, Brazil

Keico Tanaka,[1,4]* Norihiko Itoh,[1] Waka Saitoh-Inagawa,[1] Eiichi Uchio,[1] Satoshi Takeuchi,[1] Koki Aoki,[2] Eduardo Soriano,[3] Mauro Nishi,[3] Rubens Belfort Júnior,[3] Charlotte M. Hársi,[4] Liping Tsuzuki-Wang,[4] Edison L. Durigon,[4] Klaus E. Stewien,[4] and Shigeaki Ohno[1]

[1]*Department of Ophthalmology, Yokohama City University School of Medicine, Yokohama, Japan*
[2]*Aoki Eye Clinic, Sapporo, Japan*
[3]*Department of Ophthalmology, Universidade Federal de São Paulo, São Paulo, Brazil*
[4]*Laboratory of Clinical and Molecular Virology, Department of Microbiology, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil*

Genome analysis was carried out on adenovirus strains isolated from patients with acute follicular conjunctivitis in the city of São Paulo, Brazil. Eighteen conjunctival scrapings, collected between December 1993 and March 1994, were analyzed by two methods: a combination of polymerase chain reaction with restriction fragment length polymorphism and viral DNA restriction analysis, carried out using 10 restriction endonucleases: *Bam*HI, *Bgl*I, *Bgl*II, *Hin*dIII, *Kpn*I, *Sac*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I. Among 11 adenovirus detected by cell culture isolation, nine were Ad8, and two were Ad7. By restriction analysis the Ad8 isolates were typed as two new variants–Ad8/D11 (seven of nine samples) and Ad8/D12 (two of nine samples). Ad7 isolates were identified as a subtype of the widespread genome type Ad7b and the virulent type Ad7h, a predominant genome type circulating in Argentina, Chile, and Uruguay but absent in Brazil until 1991. ***J. Med. Virol. 61:143–149, 2000.***

KEY WORDS: Brazil; acute conjunctivitis; adenovirus; new genome types; polymerase chain reaction with restriction fragment length polymorphism; genome typing

INTRODUCTION

Since adenoviruses were first cultured and reported as distinct viral agents in 1953 [Rowe et al., 1953], 49 serotypes of human adenovirus have been described in the literature [Schnurr and Dondero, 1993]. They are classified into six subgenera, A to F, according to their biologic, immunologic, and biochemical properties [Wadell et al., 1986]. Among them, adenovirus types 3, 4, 7, 8, 11, 19, and 37 are reported worldwide as main causative agents of viral conjunctivitis [Wadell et al., 1986; Sawada et al., 1987; Gomes et al., 1988; Itoh et al., 1999]. Serotype 7 is associated with respiratory and ocular diseases, showing great genetic variability. Twenty-six genome types of Ad7 have been identified, including the genome type Ad7h, from cases of severe lower respiratory tract diseases isolated in Argentina, Chile, and Uruguay [Wadell et al., 1985; Bailey and Richmond, 1986; Li and Wadell, 1986; Kannemeyer et al., 1988; Golovina et al., 1991; Niel et al., 1991; Kajon et al., 1996; Azar et al., 1998]. Serotype 8, isolated for the first time in 1955, is the original causative agent of epidemic keratoconjunctivitis [Jawetz et al., 1955]. So far, Ad8 genome types from A to F [Fujii et al., 1983, 1984] and from Ad8/D1 to Ad8/D10 [Adrian et al., 1990; De Jong et al., 1992] have been described. The genome type Ad8/D1 is, by definition, the DNA variant to which the prototype strain Trim belongs. Although distinct nomenclature systems were used for these genome types, some of them are coincident.

The importance of adenoviruses as causative agents of acute conjunctivitis is not well established in South America. The only study concerning this topic was carried out in two different areas in Brazil, Belém and Rio de Janeiro, and the isolated adenovirus strains were identified as variant Ad4a. This variant was associated with pharyngoconjunctival fever and acute respiratory disease [Gomes et al., 1988]. The ethnic and geographic diversity and the high migration rate observed in Brazil may facilitate dispersion and propagation of these viruses and the appearance of new variants in areas

*Correspondence to: K. Tanaka, Department of Ophthalmology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
E-mail: yokogi@ma2.justnet.ne.jp
Accepted 5 October 1999

with high population density. This study reports the detection and genetic characterization of adenovirus strains isolated from patients with acute follicular conjunctivitis in the city of São Paulo, Brazil.

MATERIALS AND METHODS

Clinical Specimens

Between December 1993 and March 1994, conjunctival swabs from eighteen 25- to 40-year-old patients with acute follicular conjunctivitis were collected in the eye clinic of the Department of Ophthalmology, Universidade Federal de São Paulo, located in the city of São Paulo, Brazil. The clinical materials were collected by scraping conjunctiva with a sterile swab, transported to the laboratory in minimum essential medium supplemented with 0.5% fetal bovine serum plus antibiotics and antifungal agents, and stored at –20°C. The work was carried out in the Department of Ophthalmology, Yokohama City University School of Medicine, Japan.

Detection of Adenoviruses by Polymerase Chain Reaction

Adenoviruses were detected in samples using a polymerase chain reaction (PCR) method for adenoviruses described by Saitoh-Inagawa [1996]. For the preparation of DNA, 200 μL of the clinical sample was centrifuged for 30 min at 12,000 × g. DNA was extracted from the pellet using 98 μL of lysis buffer (10 mM Tris-HCl at pH 8.3, 1 mmol/L EDTA, 0.5% Tween 20) and 2 μL of proteinase K (200 μg/mL). The mixture was incubated for 1 hr at 55°C, and then the solution was heated for 10 min at 95°C. The PCR was performed in two steps designated first PCR and nested PCR. The first PCR amplifies a fragment of 1,004 bp of the hexon gene, and the nested PCR amplifies a fragment of 956 bp using DNA amplified in the first PCR as template. The pair of primers of the first PCR, designated AdTU7 and AdTU4′, recognize the sequence 5′-GCCACCTTC-TTCCCCATGGC-3′ (position 20734 to 20753) and 5′-GTAGCGTTGCCGGCCGAGAA-3′ (position 21718 to 21737), respectively. The pair of primers for the nested PCR, designated AdnU-S′ and AdnU-A, recognize the sequences 5′-TTCCCCATGGCNCACAACAC-3′ (position 20743 to 20762) and 5′-GCCTCGATGACGCCGC-GGTG-3′ (position 21679 to 21698), respectively. The PCR was carried out in a Cetus 9600 thermal cycler (Perkin Elmer Cetus, Emeryville, CA) using a cycle consisting of 94°C for 1 min (denaturation), 50°C for 1 min (annealing), and 72°C for 2 min (primer extension) for 36 cycles. After the last cycle, the DNA was extended at 72°C for 7 min.

Identification of Adenoviruses by Polymerase Chain Reaction–Restriction Fragment Length Polymorphism

Approximately 1 μg of DNA amplified by nested PCR was digested with 5 U of the restriction endonucleases *Eco*T14I, *Hae*III, and *Hin*fI under conditions specified by the manufacturer (Takara Shuzo Co., Kyoto, Japan). The digested fragments were loaded onto 5% polyacrylamide gel and run for approximately 40 min at 100 V in Tris-borate buffer at pH 8.0 with 1 mmol/L EDTA. After staining with ethidium bromide (1 μg/mL), the bands were made visible under an ultraviolet transilluminator and photographed with a Polaroid camera. The 100-bp DNA ladder (Gibco BRL, Rockville, Maryland) was used as molecular weight standard. The adenovirus type was determined by comparison of the resulting restriction patterns with those described in the literature [Saitoh-Inagawa et al., 1996], and then new electrophoresis was conducted by loading DNA of the samples and the respective prototypes on the same gel.

Virus Isolation

HEp-2 cells were used for virus isolation and propagation for DNA extraction. Clinical specimens were inoculated into culture tubes containing a subconfluent monolayer of cells and incubated at 35°C with medium changes at intervals of 3 or 4 days. The cells were examined daily until the appearance of cytopathic effects.

DNA Extraction for DNA Restriction Analysis

DNA was extracted following the protocol described by Wadell and De Jong [1980], with some modifications. Cultures of HEp-2 cells in 25-cm^2 flasks were inoculated with adenovirus stocks and incubated at 35°C until 75–100% of the cells exhibited cytopathic effects. The cells were pelleted and rinsed twice with phosphate-buffered saline and then suspended in 1 mL of Hirt lysis solution (10 mmol/L Tris, 1 mmol/L EDTA, 0.6% sodium dodecyl sulfate at pH 8). Proteinase K was added to a final concentration of 50 μg/mL, and the samples were incubated at 37°C for 1 hr. Cellular DNA was precipitated with NaCl (1 mol/L) overnight at 4°C and discharged. The supernatant was cleaned with a mixture of ribonucleases A and T1, proteinase K (200 μg/mL), and phenol–chloroform extraction. Viral DNA was precipitated with sodium acetate (final concentration of 0.3 mol/L) and ethanol and suspended in 50 μL of TE buffer (10 mmol/L Tris-HCl at pH 8.0 and 1 mmol/L EDTA).

DNA Restriction Analysis

Aliquots of 2 μL of viral DNA (approximately 1 μg) were digested with 5 U of restriction endonucleases *Bam*HI, *Bgl*I, *Bgl*II, *Hin*dIII, *Kpn*I, *Sac*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I under conditions specified by the manufacturer (Takara Shuzo Co.). Restriction enzyme digests were loaded onto 1.2% agarose gels and run for 2 hr at 100 V in Tris-acetate buffer (pH 8.0) with 1 mmol/L EDTA. After staining with ethidium bromide (1 μg/mL), the fragments were made visible under an ultraviolet transilluminator and photographed with a Polaroid camera. *Eco*T14I and *Hin*dIII digests of lambda DNA (Takara Shuzo Co.) and a 1-kb DNA ladder (Gibco BRL) were run as molecular weight standard. Subgenus and serotype identifications were undertaken by comparison of the resulting patterns with

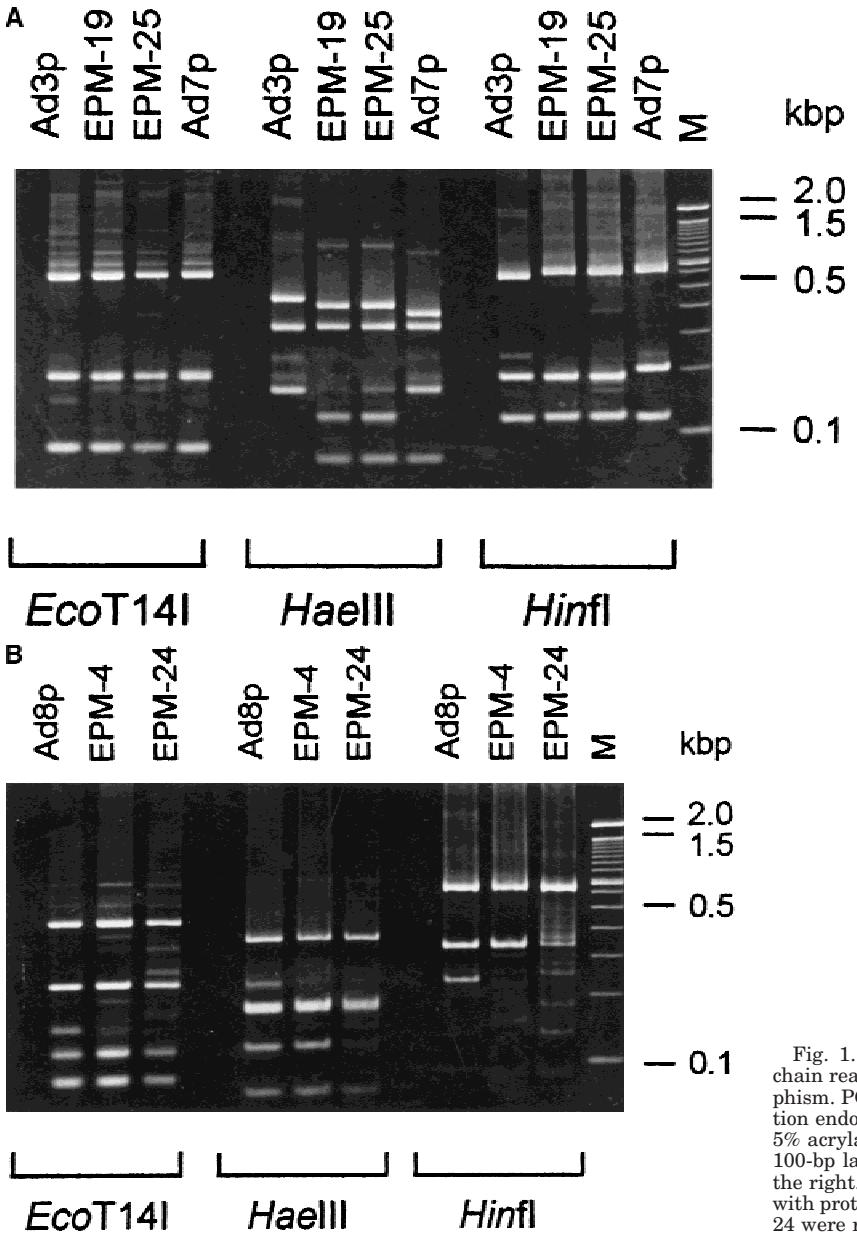

Fig. 1. Characterization of adenovirus by polymerase chain reaction (PCR)–restriction fragment length polymorphism. PCR-amplified products were digested with restriction endonucleases *Eco*T14I, *Hae*III, and *Hin*fI and run in 5% acrylamide gel. The molecular weight marker (M) is a 100-bp ladder. The sizes of the fragment are indicated on the right. **A**: Strains EPM-19 and EPM-25 were run along with prototypes Ad3 and Ad7. **B**: Strains EPM-4 and EPM-24 were run along with prototype Ad8.

the published restriction patterns of the prototypes and genome types [Fujii et al., 1983, 1984; Adrian et al., 1986; Bailey and Richmond, 1986; Li and Wadell, 1986; Kannemeyer et al., 1988; Adrian et al., 1990; Golovina et al., 1991; Niel et al., 1991; De Jong et al., 1992; Kajon et al., 1996; Azar et al., 1998].

RESULTS

Adenovirus Detection by Polymerase Chain Reaction and Culture Isolation

Adenoviruses were detected in 12 of 18 (66%) patients with acute follicular conjunctivitis by PCR and isolated in HEp-2 cells from 11 (61%) patients. The results obtained by culture isolation were in agreement with those obtained by PCR, with the difference that PCR detected one strain more.

Polymerase Chain Reaction–Restriction Fragment Length Polymorphism

The DNA products of 12 adenoviruses amplified by PCR were digested by restriction endonucleases *Eco*T14I, *Hae*III, and *Hin*fI, and the digested fragments were electrophoresed on 5% acrylamide gel along with the prototypes. Two strains detected in the city of São Paulo (EPM-19 and EPM-25) were identified as an intermediate of Ad3 and Ad7 (Ad3/7). The digested fragments of these strains were run along with Ad3 and Ad7 prototypes. With *Eco*T14I they showed the same patterns of Ad3 and Ad7 prototypes, but with *Hae*III and *Hin*fI neither were identical to the prototypes (Fig.1A). All other strains, represented in the figure by the strains EPM-4 and EPM-24, showed pat-

TABLE I. Description of the Genome Types Isolated

Serotype	Genome type	Strain	Number of isolates	Enzyme code[c]
Ad7	Ad7b[a]	EPM-19	1	
Ad7	Ad7h[a]	EPM-25	1	
Ad8	Ad8/D11[b]	EPM-4	7	1 4 3 1 1 1 3 1
Ad8	Ad8/D12[b]	EPM-24	2	1 4 1 1 1 1 3 1

[a]Nomenclature system suggested by Li and Wadell, 1986.
[b]Nomenclature system suggested by Adrian et al., 1985; new genome types.
[c]Restriction endonucleases in alphabetical order: *Bam*HI, *Bgl*I, *Bgl*II, *Hin*dIII, *Kpn*I, *Sac*I, *Sal*I, and *Sma*I.

terns similar to the Ad8 prototype with all restriction endonucleases used (Fig.1B).

DNA Restriction Analysis

The analysis of adenovirus strains isolated in the city of São Paulo, Brazil, was carried out initially using restriction endonucleases *Bam*HI, *Bgl*II, *Hin*dIII, and *Sma*I. In order to compare our strains to those described in the literature, further analysis was done by restriction with *Sal*I, *Xba*I, and *Xho*I (for Ad7 strains) and *Bgl*I, *Kpn*I, *Sac*I, and *Sal*I (for Ad8 strains). The description of the genome types is presented in Table I. The strains typed as Ad3/7 by PCR–restriction fragment length polymorphism (RFLP) were identified as being two distinct genome types of Ad7. The strain EPM-19 exhibited restriction patterns similar to those of Ad7b with *Bam*HI, *Bgl*II, *Hin*dIII, *Sma*I, *Xba*I, and *Xho*I. A deviant profile was observed with *Sal*I. The strain EPM-25 exhibited restriction patterns identical to those of Ad7h with all restriction endonucleases (Fig. 2A,B). Nine isolates, typed as Ad8 by PCR-RFLP, were confirmed as Ad8 and divided in two new genome types by DNA restriction analysis. Seven isolates, represented in the figures by the strain EPM-4, showed restriction patterns similar to those of Ad8/D1 with *Bam*HI, *Hin*dIII, *Kpn*I, *Sac*I, and *Sma*I. The restriction pattern with *Bgl*I was similar to that of Ad8/D10 and with *Sal*I was similar to that of Ad8/D5. A deviant pattern, different from that described previously, was noted with *Bgl*II. Two isolates, represented in the figures by the strain EPM-24, shared restriction patterns similar to Ad8/D1 with *Bam*HI, *Bgl*II, *Hin*dIII, *Kpn*I, *Sac*I, and *Sma*I; with enzymes *Bgl*I and *Sal*I, however, they displayed profiles similar to those of Ad8/D10 and Ad8/D5, respectively (Fig. 3A,B).

DISCUSSION

This study reports the detection of adenovirus serotypes Ad7 and Ad8 in cases of acute follicular conjunctivitis in the city of São Paulo, Brazil. Adenoviruses were found in the majority of the cases examined (66%); among them, Ad8 was the most common. These results are in agreement with those obtained in Karachi, Pakistan, where adenoviruses were isolated in 75% of patients with acute follicular conjunctivitis [Woodland et al., 1992]. Nine isolates were recognized by PCR-RFLP as Ad8. The high incidence of Ad8 found in the present study is in agreement with findings of other reports, in which Ad8 was the most common causative agent of adenovirus ocular infection [Ishii et al., 1987; Mahafzah and Landry, 1994]. In restriction enzyme analysis using eight enzymes, the isolates were found to be different from those described previously [Adrian et al., 1990; De Jong et al., 1992]. They were divided in two new genome types, named Ad8/D11 and Ad8/D12 according to the nomenclature system proposed by Adrian et al. [1985]. Studies in DNA homologies of serotype 8 are often difficult, owing to its propagation in cell cultures. There are strains that grow well and others that replicate slowly in the laboratory, as reported in early studies [Wigand et al., 1983; Guo et al., 1988]. In this study, the analysis of small fragments with molecular weights lower than 1 kbp was not possible because of the insufficiency of DNA. A new procedure using a combination of PCR and sequence analysis, developed by Takeuchi et al. [1999], provides detailed information about changes that have occurred in the virus, without propagation in cell cultures. This method may become an important tool in the analysis of fastidious serotypes, to which DNA restriction analysis is not applicable.

Two isolates were identified as type Ad3/7 by PCR-RFLP; they were later submitted to confirmatory testing with seroneutralization, resulting in cross-reaction between serotypes 3 and 7. DNA restriction analysis of these isolates resulted in the recognition of the genome types Ad7b and Ad7h. It is known that Ad3 and Ad7 are very closely related serotypes belonging to subgenus B. Restriction profiles with some enzymes are shared by these two serotypes [Bailey and Richmond, 1986; Niel et al., 1991], and sequence analysis of the E3 region and fiber gene has shown that Ad7h is an intermediate strain 7-3 [Kajon and Wadell, 1996]. Genome type Ad7b was first described in 1978 [Wadell and Varsanyi, 1978] and is considered to be a virulent type causing serious or fatal respiratory disease in infants [Wadell et al., 1980]. Genome type Ad7h was isolated for the first time in 1984 in Argentina [Kajon and Wadell, 1992] and is the predominant type circulating since 1986 in Argentina, Chile, and Uruguay [Kajon et al., 1996]. This genome type has been associated with severe acute lower respiratory tract disease in infants, with fatal outcomes. In Brazil, early studies in molecular epidemiology isolated Ad7e as the only genome type in two cities: Belém and Rio de Janeiro [Wadell et al., 1985]. Genome type Ad7b was detected by Gomes et al. [1989] in fecal samples collected from children during an epidemiological survey in the city of São Paulo. Later, Hársi et al. [1995] found Ad7b to be a unique genome type of Ad7, isolated from cases of bronchopneumonia and diarrhea in fecal samples collected in São Paulo between 1988 and 1989. In Rio de Janeiro, Moraes et al. [1997], analyzing 40 isolates of Ad7, found the genome type Ad7e from 1980 to 1983 and the genome type Ad7b from 1984 to 1991 as the predominant type. None of these studies detected the genome type Ad7h.

In the present study, the strain EPM-19 was identi-

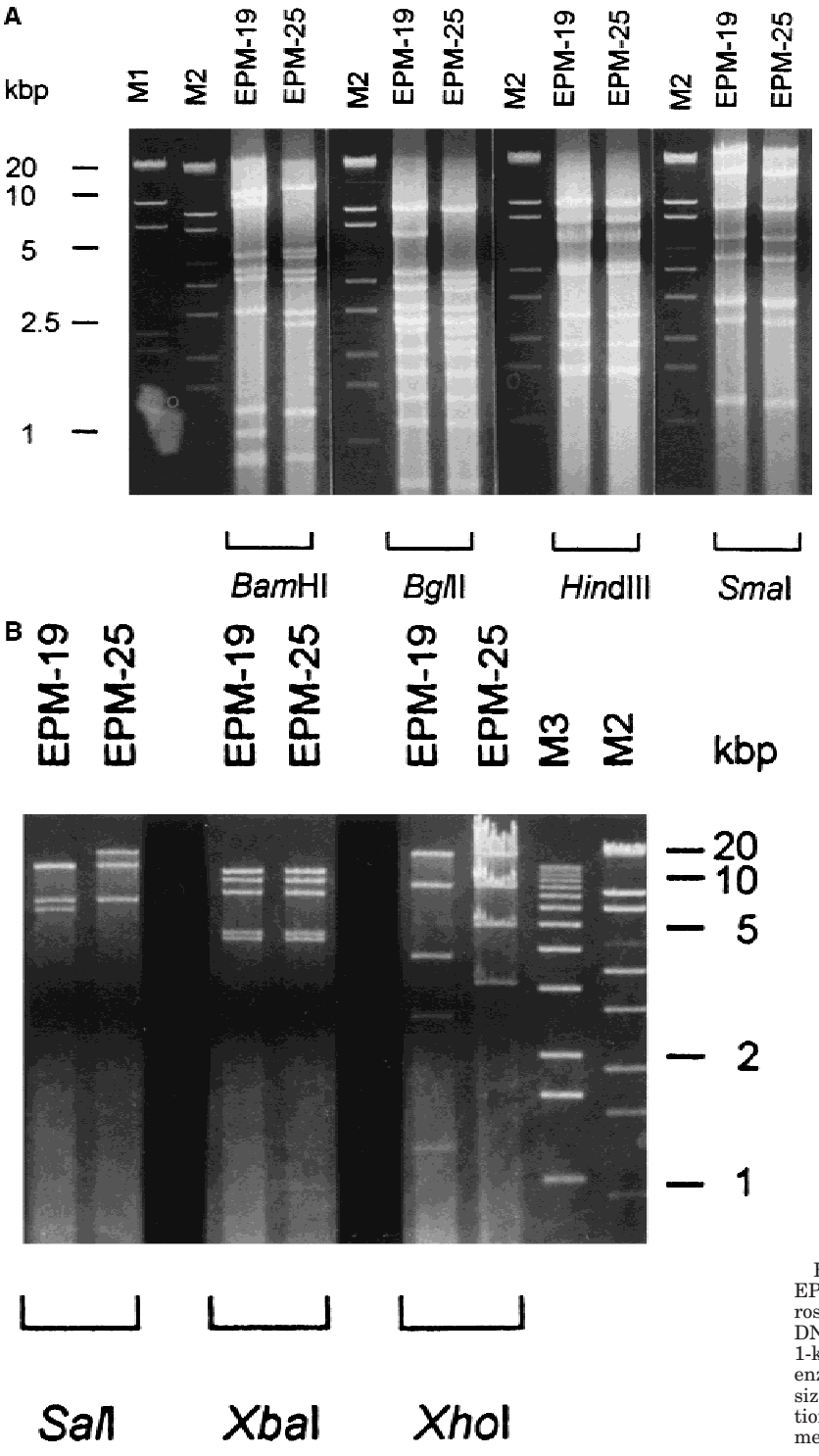

Fig. 2. Restriction patterns of strains EPM-19 and EPM-25. Restriction fragments were run in 1.2% agarose gel using as molecular weight markers the lambda DNA digested with *Hin*dIII (M1), *Eco*T14I (M2), and a 1-kb DNA ladder (M3). **A**: Restriction patterns with enzymes *Bam*HI, *Bgl*II, *Hin*dIII, and *Sma*I. Fragment sizes are indicated at the left side of the gel. **B**: Restriction patterns with enzymes *Sal*I, *Xba*I, and *Xho*I. Fragment sizes are indicated at the right side of the gel.

fied as Ad7b, but the *Sal*I profile was deviant. It is possible that this strain is one of the subtypes Ad7b1, Ad7b2, or Ad7b3 described by Bailey and Richmond [1986] or even a new subtype. The strain EPM-25 exhibited restriction patterns identical to Ad7h with all restriction endonucleases. Although the number of patients is relatively small, these results compare to previous data, suggesting that the genome type Ad7h probably reached Brazil between 1991 and 1993. This finding is relevant because it indicates the spread of this virulent type from the south of the continent to the southeastern region of Brazil.

The isolation of virulent types as Ad7b and Ad7h is epidemiologically and medically important, alerting health care professionals to possible severe disease outbreaks in this country. Moreover, it points to the risk of

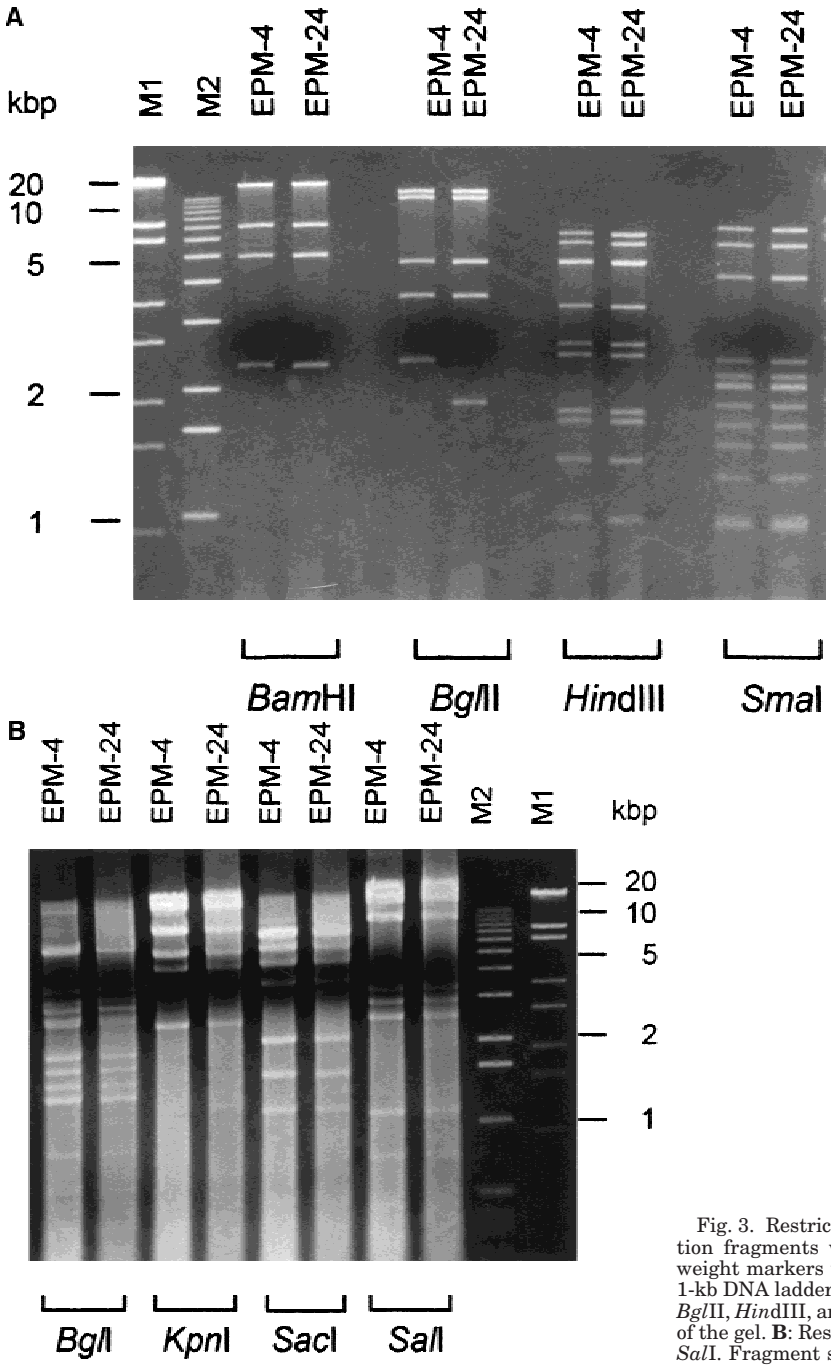

Fig. 3. Restriction patterns of strains EPM-4 and EPM-24. Restriction fragments were run in 1.2% agarose gel using as molecular weight markers the lambda DNA digested with *Eco*T14I (M1) and a 1-kb DNA ladder (M2). **A**: Restriction patterns with enzymes *Bam*HI, *Bgl*II, *Hin*dIII, and *Sma*I. Fragment sizes are indicated at the left side of the gel. **B**: Restriction patterns with enzymes *Bgl*I, *Kpn*I, *Sac*I, and *Sal*I. Fragment sizes are indicated at the right side of the gel.

replacement of the circulating strains by the highly pathogenic genome type Ad7h, which also is capable of inducing acute conjunctivitis. Since different serotypes have new variants, the continued investigation of these variants may define evolutionary tendencies of adenoviruses.

ACKNOWLEDGMENTS

We thank Hiroaki Ishiko and Akira Oshima, from Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo, for kindly providing the prototype strains of Ad3, Ad7, and Ad8 and Akemi Ikeda for her support and skillful technical assistance.

REFERENCES

Adrian T, Best B, Wigand R. 1985. A proposal for naming adenovirus genome types, exemplified by adenovirus type 6. J Gen Virol 66: 2685–2691.

Adrian T, Wadell G, Hierholzer JC, Wigand R. 1986. DNA restriction analysis of adenovirus prototypes 1 to 41. Arch Virol 91:277–290.

Adrian T, Wolf U, Lauer HG, Wigand R. 1990. Restriction site mapping of adenovirus type 8 genome types. Res Virol 141:611–624.

Azar R, Varsano N, Mileguir F, Mendelson E. 1998. Molecular epide-

miology of adenovirus type 7 in Israel: identification of two new genome types, Ad7k and Ad7d2. J Med Virol 54:291–299.

Bailey AS, Richmond SJ. 1986. Genetic heterogeneity of recent isolates of adenovirus types 3, 4, and 7. J Clin Microbiol 24:30–35.

De Jong JC, Demazure M, Legrand-Quillien MC, Le Lay G, Colin J, Wermenbol AG, Verweij-Uyterwaal MW, van der Avoort HGAM, Chastel C. 1992. New developments in the molecular epidemiology of adenovirus 8 keratoconjunctivitis. J Med Virol 38:102–107.

Fujii S, Nakazono N, Sawada H, Ishii K, Kato M, Aoki K, Ohtsuka H, Fujinaga K. 1983. Restriction endonuclease cleavage analysis of adenovirus type 8: two new subtypes from patients with epidemic keratoconjunctivitis in Sapporo, Japan. Jpn J Med Sci Biol 36: 307–313.

Fujii S, Nakazono N, Ishii K, Lin CC, Sheu MM, Chen CW, Fujinaga K. 1984. Molecular epidemiology of adenovirus type 8 (Ad8) in Taiwan: four subtypes recovered during the period of 1980–1981 from patients with epidemic keratoconjunctivitis. Jpn J Med Sci Biol 37:161–169.

Golovina GI, Zolotaryov FN, Yurlova TI. 1991. Sensitive analysis of genetic heterogeneity of adenovirus types 3 and 7 in the Soviet Union. J Clin Microbiol 29:2313–2321.

Gomes SA, Gabbay YB, Nascimento JP, Niel C. 1988. Genome analysis of adenovirus 4a, a causative agent of pharyngoconjunctival fever and respiratory diseases in Brazil. J Med Virol 26:453–459.

Gomes SA, Candeias JAN, Monteiro SP, Pereira HG, Niel C. 1989. New genome types of adenovirus types 1, 3 and 5 isolated from stools of children in Brazil. J Clin Microbiol 27(5):1022–1026.

Guo DF, Shinagawa M, Aoki K, Sawada H, Itakura S, Sato G. 1988. Genome typing of adenovirus strains isolated from conjunctivitis in Japan, Australia and the Philippines. Microbiol Immunol 32: 1107–1118.

Hársi CM, Rolim DP, Gomes AS, Gilio AE, Stewien KE, Baldacci ER, Candeias JAN. 1995. Adenovirus genome types isolated from stools of children with gastroenteritis in São Paulo, Brazil. J Med Virol 45:127–134.

Ishii K, Nakazono N, Fujinaga K, Fugii S, Kato M, Ohtsuka H, Aoki K, Chen CW, Lin CC, Sheu MM, Lin KH, Oum BS, Lee SH, Chun CH, Yoshii T, Yamazaki S. 1987. Comparative studies on aetiology and epidemiology of viral conjunctivitis in three countries of East Asia—Japan, Taiwan and South Korea. Int J Epidemiol 16:98–103.

Itoh N, Tanaka K, Aoki K, Hinokuma R, Nakagawa H, Takeuchi S, Uchio E, Shiao S, Ohno S. 1999. Four new genotypes of adenovirus type 3 isolated from patients with conjunctivitis in Japan. J Med Virol 59:73–77.

Jawetz E, Kimura SJ, Hanna L, Coleman VR, Thygeson P, Nicholas A. 1955. Studies on the etiology of epidemic keratoconjunctivitis. Am J Ophthalmol 40:200–211.

Kajon AE, Wadell G. 1992. Molecular epidemiology of adenoviruses associated with acute lower respiratory disease of children in Buenos Aires, Argentina (1984–1988). J Med Virol 36:292–297.

Kajon AE, Wadell G. 1996. Sequence analysis of the E3 region and fiber gene of human adenovirus genome type 7h. Virology 215: 190–196.

Kajon AE, Mistchenko AS, Videla C, Hortal M, Wadell G, Avendano LF. 1996. Molecular epidemiology of adenovirus acute lower respiratory infections of children in the South Cone of South America (1991–1994). J Med Virol 48:151–156.

Kannemeyer JR, Brooks LA, Dumbell KR, Keen GA. 1988. Two new genome types of adenovirus 7c. J Med Virol 24:101–108.

Li QG, Wadell G. 1986. Analysis of 15 different genome types of adenovirus type 7 isolated on five continents. J Virol 60:331–335.

Mahafzah A, Landry ML. 1994. Restriction endonuclease analysis of adenovirus isolates from sporadic and epidemic ocular infections: experience in a clinical laboratory. J Med Microbiol 40:385–388.

Moraes MT, Leite JP, Siqueira MM, Portes AS, Krawczuk MM, Nascimento JP. 1997. Genomic characterization of adenovirus serotype 7 isolated in Brazil from acute respiratory disease patients during the period from 1980 to 1991. Rev Inst Med Trop São Paulo 39(4):185–189.

Niel C, Moraes MTB, Mistchenko AS, Leite JPG, Gomes SA. 1991. Restriction site mapping of four genome types of adenovirus types 3 and 7 isolated in South America. J Med Virol 33:123–127.

Rowe WP, Huebner RJ, Gillmore LK, Parrot RH, Ward TG. 1953. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc Soc Exp Biol Med 84:570–573.

Saitoh-Inagawa W, Oshima A, Aoki K, Itoh N, Isobe K, Uchio E, Ohno S, Nakajima H, Hata K, Ishiko H. 1996. Rapid diagnosis of adenoviral conjunctivitis by PCR and restriction fragment length polymorphism analysis. J Clin Microbiol 34:2113–2116.

Sawada H, Aoki K, Kawana R, Matsumoto I, Shinagawa M, Guo DF, Fajardo FU. 1987. Molecular epidemiology of adenovirus conjunctivitis in Sapporo, Japan, and Manila, the Philippines. Jpn J Ophthalmol 31:538–546.

Schnurr D, Dondero ME. 1993. Two new candidate adenovirus serotypes. Intervirology 36:79–83.

Takeuchi S, Itoh N, Uchio E, Aoki K, Ohno S. 1999. Serotyping of adenoviruses on conjunctival scrapings by PCR and sequence analysis. J Clin Microbiol 37:1830–1845.

Wadell G, Varsanyi TM. 1978. Demonstration of three different subtypes of adenovirus type 7 by DNA restriction site mapping. Infect Immun 21:238–246.

Wadell G, De Jong JC. 1980. Restriction endonucleases in identification of a genome type of adenovirus 19 associated with keratoconjunctivitis. Infect Immun 27:292–296.

Wadell G, Varsanyi TM, Lord A, Sutton RNP. 1980. Epidemic outbreaks of adenovirus 7 with special reference to the pathogenicity of adenovirus genome type 7b. Am J Epidemiol 112:619–628.

Wadell G, Cooney MK, Linhares AC, Silva L, Kennett ML, Kono R, Gui-Fang R, Lindman K, Nascimento JP, Schoub BD, Smith CD. 1985. Molecular epidemiology of adenoviruses: global distribution of adenovirus 7 genome types. J Clin Microbiol 21:403–408.

Wadell G, Allard A, Evander M, Li QG. 1986. Genetic variability and evolution of adenoviruses. Chemica Scripta 26B:325–335.

Wigand R, Gelderblom H, Ozel M, Distler H, Adrian T. 1983. Characteristcs of mastadenovirus h8, the causative agent of epidemic keratoconjunctivitis. Arch Virol 76:307–319.

Woodland RM, Darougar S, Thaker U, Cornell L, Siddique M, Wania J, Shah M. 1992. Causes of conjunctivitis and keratoconjunctivitis in Karachi, Pakistan. Trans R Soc Trop Med Hyg 86:317–320.